PRIAR using a Graph Segmentation Method

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Abstract: Recently, we have suggested a simple and general-purpose method able to combine high-resolution analysis with the classification and identification of components of microscopy imaging. The method named PRIAR (Pattern Recognition Image Augumented Resolution) is a tool developed by the authors that gives the possibility to enhance spatial and photometric resolution of low-res images. The implemented algorithm follows the scheme: 1) image classification; 2) blind super-resolution on single frame; 3) pattern-analysis; 4) reconstruction of the discovered pattern.
In this paper, we suggest some improvements of the PRIAR algorithm, in particular, the definition of a segmentation method which is based on homomorphism between a processed image and a graph describing the image itself, able to identify object of interest in complex patterns. The case study is the identification of organs inside biological cells acquired with Atomic Force Microscopy Technique.

1 INTRODUCTION

Microscope image analysis is used in many fields of technology, science and medicine. In all these applications, a common problem is represented by the low resolution of objects of interest, so that detection and classification are difficult and, because of correspondent poor resolution, analysis of such objects can be lead to misunderstanding. As a consequence, pattern recognition (PR) and identification of objects of interest is a first necessary step in order to improve the quality of image analysis, the second step is to improve the resolution from low level to high level, or equivalently, multiple scale resolution.

PR applied to the imaging techniques is a term denoting supervision methods developed by the combination of machine learning, artificial intelligence and data mining. PR methods can be categorized according to the type of learning procedure used to generate output value from input images. For example, supervised learning procedure assumes that a set of training data has been previously provided (Gonzalez and Woods, 2008). Then, a further learning processing generates a model that attempts to meet two general requests: i), perform as well as possible on the training data, and, ii) generalize as well as possible to new data or other input images. The other one category is represented by the unsupervised learning methods. This class of pattern recognition methods assumes training data that has not been previously labelled, and try to find inherent patterns in the data that can be used to identify a correct output value for new data instances (Theodoridis et al., 2008). A combination of the two categories is also possible, mixing labelled and unlabeled data in suitable ways, depending by the data in input, and where the data to be labelled is the training data (Bishop, 2006). Most common pattern recognition algorithms are probabilistic in nature, in that they use statistical inference to find label for a given instance. In many applications, many probabilistic algorithms output a list of n-best labels with associated probabilities, instead of simply best label. For example, PR can be used for classification, which attempts to assign each input value to one of a given set of classes, in this case, the n-best labels should be fairly small (a binary choice for n is the best, yes or not, 1 or 0, etc). All the microscopic techniques require highly sophisticated pattern recognition supervised methods, for example PR is crucial for the segmentation procedure of components to be identified and classified in a large image. Since PR strategies are strongly dependent by the application, we focused

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the attention on methods connected to the identification or organs and components inside biological systems like animal cells. For example, systemic analysis of subcellular protein localization provides a key for understanding gene functions and physiological condition of the cells. However, recognition of cell images of subcellular structures highly depends on experience and becomes the rate-limiting step when classifying subcellular protein localization. Several research groups have extracted specific numerical features for the recognition of subcellular protein localization, but these recognition systems are restricted to images of single particular cell line acquired by one specific imaging system and not applied to recognize a range of cell image sources.

Our imaging technique is based on the scanning probe microscope (DAcunto, 2011), a technique that measures the cells touching them with a probe approaching a resolution on the nanometer scale. On the contrary, with respect to other microscope technique, like for example, the confocal microscopy, the interaction between the microscopic tip probe and the sample is limited to the cell cytoskeleton, i.e., the cell surface, so pattern recognition methods must be addressed to identify skeleton components, like microtubules, microfilaments, intermediate filaments, etc. Once a skeleton components is identified, a procedure of super-resolution (enhanced resolution) could improve the data analysis and mining. The problem of enhancing resolution of a single image refers to the task of obtaining a high-resolution enlargement of a single low resolution image, commonly recognised as single-frame super-resolution problem. This problem is intrinsically ill-posed because are generally multiple frame images that can be combined to obtain final high-resolution images. Accordingly to enhancing resolution for a single image, strong prior information must be realized. This information is available either in the explicit form of a distribution defined on an image class, or an implicit form of an example image which leads to example-based super-resolution image (Kim and Kwon, 2010). One diffuse approach for the super-resolution of low-resolution image has been characterized by algorithms including nearest neighbour (NN)-based estimation. In such approach, there are two distinct steps, in the first one, pairs of low-resolution and corresponding high-resolution image patches are collected. Then in the second one, the super-resolution step, each patch of the given low-resolution is contrasted to the stored low-resolution patches, and the high-resolution patch corresponding to the nearest low-resolution patch and satisfying a certain spatial neighbourhood similarity can be evidenced so that can be considered as the final output of the super-resolution image.

2 PRIAR 1.0: BASIC FUNCTIONALITIES

The PRIAR (Pattern Recognition Image Augumented Resolution) is a tool we have developed that gives the possibility to enhance the resolution of low resolution images. It enhances the spatial resolution and the depth of the image. The implemented algorithm follows the scheme: 1) image classification; 2) blind super-resolution on single frame; 3) pattern-analysis; 4) reconstruction of the discovered pattern. PRIAR runs on Matlab 2013b, and the main features of the computer used to test the program follows:CPU Intel(R) Core(TM) i5-2400 CPU @ 3.10GHz (4 cores) with a L2 cache of 6144 KB with 16GB of RAM. The program has been developed as mono-task, the idea is to have a pipe-flow where each piece calculates a subproblem sequentially. PRIAR works with in input a low-resolution image and produces in output a high-resolved computed image where the objects to be identified are recognized and substituted with their correspondent models. The execution time depends on the size of the input data. A typical execution time for patch images of 128×128 pixels, 8 bit per pixel, is of about 70 seconds. The occupied memory depends on the image size, the presented typical example need of about 256 MB of RAM. The Matlab code that describes algorithm implementation is detailed elsewhere (Righi, 2014), here, we resume the basic functions included inside with the following pseudo-code:

```
01 Function [path enhanced_image]=PRIAR(input_image)
02 class=classification(input_image)
03  switch class
04   case:grating
      segmentation_type=otsu
05   case:cell
      segmentation_type=edge_discover
06  end
07  sr_image=blind_sr(input_image)
08  initial_path=
     PRIAR-S(seed,super_resolved_image)
09  edge_discovered=
     edge_discover(sr_image,segmentation_type)
10  path=
     explore_extend(initial_path,segmented_image);
11  enhanced_image =build_model(sr_image,class)
12 end
```

The algorithm models a general purpose method to analyse the images, in fact it uses a modular solution that permits to extend the class of the recognized images and the image enhancement. The line 02 of the pseudo-code calls a data-mining function

that classify the input image. In this particular implementation the function distinguish only two classes of images: images representing a grating and images representing cells. The classification is important during the next stages to choose the better segmentation to adopt. The line 07 reports the function call that super-resolve the image. During this step the analysed image (that is a low resolution image) is improved by increasing the colorimetric and spatial resolution. The PRIAR implements the following SR algorithms: Kim-Kwon (Kim and Kwon, 2010), spline interpolation (Sonka et al., 2007), nearest-neighbor interpolation (Ikonen and Toivanen, 2005), bilinear interpolation (Bourke, 2001), bicubic interpolation (Getreuer, 2011), box-shaped kernel interpolation (Ardizzone, 2009), Lanczos-2 kernel interpolation (Getreuer, 2011) and Lanczos-3 kernel interpolation (Ardizzone, 2009). The line 08 describes the first step to map the object of interest (microtubule). In each case, it needs a seed. The seed can be simply a point of the object we are going to map or a polyline that follows a part of the object we are going to discover. This is the algorithm we are going to describe in detail in the next sections. The main idea of the *explore algorithm* is to crawl the surface until certain parameters are respected. The role of the parameters can be summarized in the fact that the gap between two next pixel must be enough small. At the end of this exploration we have an initial path that is a subset of the object we are going to trace. The hypothesis that the initial path is a subset of the final path is guarantee by the strong constraint that are applied by the explore algorithm. The complexity of this algorithm is linear with the number of the pixel that composed the matrix. The explore algorithm performs a local exploration so it is a local search algorithm. Line 09 shows the edge discover function call. The function used with grating images is different by the one adopted with cell images in order to avoid unwanted shadows. In fact, in order to edging the grating super-resolved image, we combine the Sobel method (Gonzalez and Woods, 2008; Gonzalez et al., 2010), the Prewitt method (Gonzalez and Woods, 2008; Gonzalez et al., 2010), the Roberts method and the Laplacian of Gauss method (Gonzalez and Woods, 2008; Gonzalez et al., 2010). If the we are analyzing a cell image, we combine the Canny method (Gonzalez and Woods, 2008; Gonzalez et al., 2010), the Zero-Cross method (Gonzalez and Woods, 2008; Gonzalez et al., 2010) and the previous listed methods.

3 PRIAR 1.1: IMPROVEMENTS OF PRIAR WITH GRAPH SEGMENTATION METHOD

In this section, we put in evidence the procedure underlining the line 08 of PRIAR algorithm: i.e., the identification of tyhe object of interest trough the segmentation based on graph approach. Image segmentation represents a challenge in image analysis. The kind of segmentation that is required depends on user objectives so different definitions and criteria have been developed for image segmentation. Here, we put in evidence a new method to segment a particular area of a gray-scale image,as previously identified inside PRIAR procedure.

We represent the image with a proximity graph where each node is associated to one image pixel. The edges are labelled and reflects the neighbour relation between pixels. Weights of edges is computed by a function based on properties of corresponding pixel such as the color intensity and the position. According to this representation the graph is used to determining the image segments. Let us consider a non-oriented weighted graph $G = (V, E, W)$ where V is the set of the vertex that represents the image pixels, E the edges that connect the vertex and the weighted edges W represent the relationships between neighbour pixels. The weight matrix w_{ij}, where $i, j \in I$ where I is the image, is a symmetric matrix. The use of the image segmentation reduces the problem to a graph clustering problem, in fact an image segment corresponds to partition of the graph: the nodes of a partition are strongly connected while the nodes that belongs to different partitions are weakly connected.

The application of a graph clustering algorithm to a proximity graph will partition it into sub-graphs, the objective of this research is to identify a single object in an image so the PRIAR 1.1 algorithm will take in account only a sub-graph. This paper extends the algorithm described in (Righi et al., 2014; DAcunto et al., 2015).

3.1 Image Representation

The graph $G = (V, E, W)$ represents the input image where each node V represents a pixel of the source image and the weight w_{ij} is the distance between two nodes. The distance between two nodes corresponds to the distance between the the pixel i and the pixel j. Since we want to recognize pixels that are in the same segment, the weight of the graph represent the color similarity and it is calculated by a likelihood function based on the local intensity of neighboring pixels.

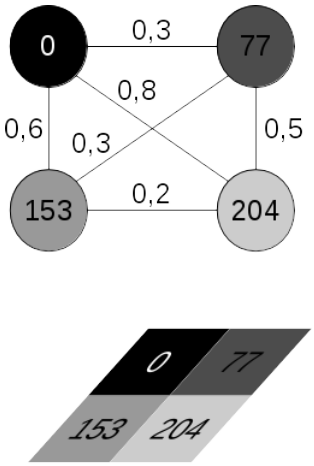


Figure 1: a portion of a 8-bit grayscale image and its associated graph.

The weight are calculated as described in function 1.

$$w_{ij} = \begin{cases} 1 - \frac{\|c_i - c_j\|}{max(c)} & \text{if } d(i,j) \leq \sqrt{2} \\ 0 & \text{otherwise} \end{cases} \quad (1)$$

where $d(i,j)$ is the Euclidean distance between pixels. There is an edge linking between two nodes only if the nodes are close together. By using the weight of function 1 next edges that have a close color belong to the same structure: in this method of segmenting a segment is determined by the homogeneity of the color of pixels that constitute it. Figure 1 shows the association between a portion of a 8-bit grayscale image and its associated graph.

Note that by using this formulation the result can be easily verified by a visual inspection of the image searching homogeneous area of pixels.

A good result for a graph-based segmentation methods depends on the translation of the color information of the source image into the graph: in order to have an easily clusterization, the colour of a segment must be well separated from the color of other segments. In order of improve the segmentation procedure, the image separation algorithm must use a weighted function that is function of the input image. This function is part of the image segmentation algorithm and it is described into the next section.

3.2 Image Segmentation Algorithm

As described in section 3.1 an image can be represented by a proximity graph that can be crawled in order to calculates a segment of interest. This method implements the well-accepted criteria (Wertheimer, 1938) to recognize a segment. The segment of interest is composed of strong bonds between the vertexes that is surrounded by a set of weak bonds between the vertexes. To find a segment in proximity graphs of image, we use the graph as described in section 3.1. The key idea is that a segment is recognizable by analysing neighbourhood relationships between objects in a particular space which reflects object similarities. In most practical problems direct analysis is unrealistic due to the high dimensionality of the space (Peng et al., 2013) and it is necessary use an heuristic approach to reduce the space where to search the goal (Russell and Norvig, 2010). Our solution linearises the search problem by reducing the algorithm complexity to $O(n)$ where n is the number of the pixel. In this method the search problem is expressed by the following relation:

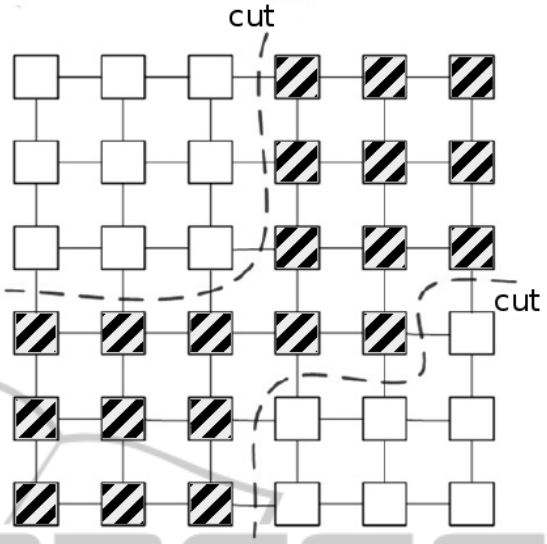


Figure 2: an example of graph cuts and the corresponding vertex weight.

$$cut(A,B) = \begin{cases} min \sum_{i,j \in A} & w_{ij} \\ max \sum_{i \in A, i \in B} & w_{ij} \end{cases} \quad (2)$$

where A is the segment we want recognize and B the background. In figure 2, we show an example of such graph cut. In order to segment the image it is used $min \sum_{i,j \in A}$ as primary objective and $max \sum_{i \in A, i \in B}$ as secondary objective. The idea to threat the segmentation as an exploring game placing a crawler (the player) on a vertex that is part of the interesting segment. The crawler can move from a vertex to another one if there exists the condition to execute this movement, the condition is a function of the input image. Considering that the weights are constant, the crawler can be implemented using a *net surfer* and a *pixel harvester*. Both net surfer and pixel harvester pro-

cedures are implemented using a deterministic finite automaton. The net surfer is modelled by a IFA (Iterated Finited Automa) (Zhang, 2008) and the pixel harvester is modelled by a DFA (Deterministic Finite Automaton) (Hopcroft and Ullman, 1969).

3.3 Net Surfer

The net surfer must navigate the graph in order to have an exhaustive search over the graph. This task is particularly important because it determines the algorithm complexity: in fact the other function have a linear complexity according to the size of the data input. In order to maintain his algorithmic complexity it is necessary that this task has an $O(n)$ complexity (Parinya et al., 2014; Zhang, 2008). The solution we illustrate respect this constraint permitting us to have a algorithm with linear complexity. The IFA is defined by $I = (S_i, S_f, S, I, A)$ where S_i is the initial state, S_f is the final state, S are the non-terminal states and A is the set of the actions. The nodes of I are homomorphic to G nodes, the operation performed by IFA are used to calculated the next node that must be examined by the pixel harvester.

The input of the net surfer is a labelled graph $G\prime = (V, E)$ where each edge has a label from 1 to 8.

The IFA we use has a number of states that is equivalent to the number of the vertex V of the G graph. Each state stores two information: the last edge that was visited and the belonging state value. The belonging state value can be U,B or $\overline{B}$:

- U means that it is a state that is not yet examined;
- B means that it is a state that corresponds to a vertex that belongs to the segment;
- $\overline{B}$ means that it is a state that does not correspond to a vertex that belongs to the segment.

We show in figure 3 the node label of the $G\prime$ graph. The net surfer gets in input the first node and mark its state as B, initializes the local variable of the node edge counter with the value 1 and initialize the variable history list of the nodes with the name of the first node. The next step consists of the examination of the variable value node edge to locate the next note to analyse (the node at the end of the edge). If the node has value U the net surfer calls the pixel harvester function (describe in sub-section 3.4) and according to the pixel harvester answer change the state of the node in B or $\overline{B}$. If the state of the node is $\overline{B}$ it will never analysed again. Otherwise the search of the nodes that are in the segment continues from this node assigning the state. Each time that a node is call back it is increased its node edge by 1 and the search

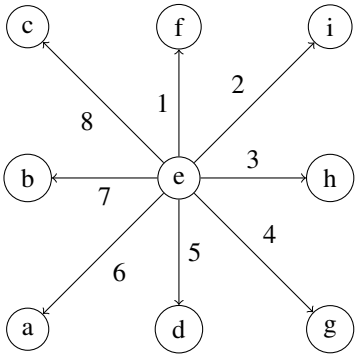
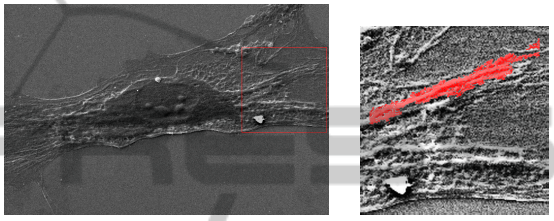


Figure 3: the labelled graph $G\prime$ used by IFA.



A B

Figure 4: (a) the box emphatize the area where it is performed the search (b) the filament tracked by PRIAR 1.1 algorithm.

starts again following the new edge. The search finishes when the variable node edge has reached the value 9 and the history list contains only one node. The returned value is a list of the pixels that are in the segment.

3.4 Pixel Harvester

The pixel harvester evaluates the distance between two pixels. Considering that ref(init) is the correspondent value of the colour intensity between the first node selected and its pixel, the adopted model consider that the distance between two adjacent nodes i and j are in the set B (the set of the nodes that are in the segment) only if the equation 3 is satisfied.

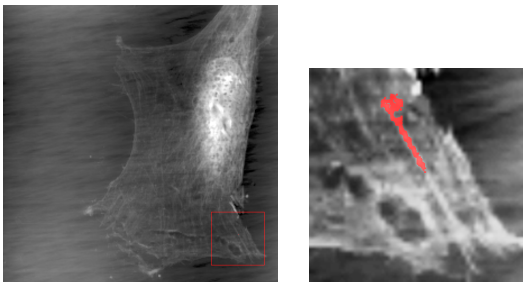
$$i \in B \wedge \left\| \mathrm{ref}\,(\mathrm{init}) - w_{ij} \right\| < v_1 \wedge w_{ij} < v_2 \Rightarrow j \in B \quad (3)$$

The constraints v_1 and v_2 depends on input data and are inserted by the user.

3.5 Additional Results

PRIAR 1.1 algorithm gives has provided interesting result during cell analysis. Here we show only two samples where we put in evidence the features of the algorithm (figures 4 and 5).

Moreover we tested our method by applying it to synthetic images containing a set of cylindrical



A B

Figure 5: (a) the box emphatize the area where it is performed the search, (b) the filament tracked by using the PRIAR 1.1 algorithm.

shapes. We found that these shapes could be recognized after both reduction of resolution and addition of noise. We found that the percentage error (number of pixels either wrongly assigned or non-assigned to the pattern to identify) was 0.8 when the signal-to-noise ratio was 11.6 dB and was 7.4 when the signal-to-noise ratio was 8.7 dB.

4 CONCLUSIONS AND FUTURE WORKS

In this paper, we have presented the PRIAR 1.1, a new improved version of the code PRIAR (Pattern Recognition Image Augmented Resolution)1.0. Essentially, the new improvement regards the identification of object of interest inside an image showing complex patterns. The application is on stem cell cytoskeleton recorded with an atomic force microscope. The key idea is that the object of interest, (for example, a segment, that in the reality represents a cytoskeleton filament) is recognizable by analysing neighbourhood relationships between objects in a particular space which reflects objects similarities. Since, in most practical problems, direct analysis is unrealistic due to the high dimensionality of the space, we improved the procedure linearizing the search problem by reducing the algorithm complexity to $O(n)$, where n is the number of the pixel.

This method shows how the use of graphs to perform pattern-recognition is a powerful tool for the recognition of objects in an image. Considering that the same object can be isomorphic with more unconnected subgraphs, we will study how to relate these subgraphs and recognize when they refer to a single object image.

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